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Chemoautotrophic Symbiosis in a Hydrothermal Vent Gastropod

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Abstract. An undescribed gastropod species collected from recently discovered deep-sea hydrothermal vents in the western Pacific contains endosymbiotic bacteria within specialized gill cells. The snails inhabit rocky vent openings where they are exposed directly to warm (2–25°C) sulfide-rich (750 μM) water emitted from the vents. The gills of this snail contain elemental sulfur and high activities of enzymes catalyzing sulfide metabolism (sulfide oxidase, ATP-sulfurylase, APS-reductase, rhodanese) and autotrophic CO_2 fixation (ribulose bisphosphate carboxylase) indicating that the bacteria function as sulfur oxidizing chemoautotrophic endosymbionts—a symbiosis described previously only in vestimentiferan and pogonophoran tubeworms, oligocheate worms, and bivalve molluscs. This represents the first documentation of chemoautotrophic potential among the numerous gastropod species found inhabiting the interface of reducing and oxidizing environments.

Introduction

Molluscs of the class Gastropoda obtain nutrition primarily as grazers, predators, or deposit feeders. In a recent expedition to the spreading center in the Mariana Back-Arc Basin, (western North Pacific 18° 11′ N, 144° 43′ W), dense communities of animals were discovered surrounding deep-sea hydrothermal vents. Unlike previously described hydrothermal vent communities in the eastern Pacific, where large vestimentiferan tube worms and bivalve molluscs are the most conspicuous organisms (Hessler and Smithey, 1983), at the Mariana vents dense concentrations of an undescribed mesogastropod predominate at rocky vent openings (Fig. 1). Measurements showed that these snails were exposed directly to emerging vent water with temperatures up to 25°C and concentrations of hydrogen sulfide of 750 μM (K. Johnson, University of California, Santa Barbara, pers. comm.).

Because many bivalve molluscs that are exposed directly to sulfide-rich water house chemoautotrophic sulfide-oxidizing bacteria as symbionts within modified gills (Felbeck *et al.,* 1981; Reid and Brand, 1986; Southward, 1986), we suspected that the snails may have evolved a similar strategy. We also considered the possibility that the snails housed methanotrophic bacteria as symbionts, a more recently discovered relationship in mytilids inhabiting vent-type communities (Childress *et al.,* 1986; Cavanaugh *et al.,* 1987). Unlike bivalves, however, which can filter large volumes of water and thereby readily provide their endosymbionts with the dissolved nutrients required for chemoautotrophy, the gastropods, with their tightly coiled shells, seemed at first unlikely candidates to house similar endosymbionts. Yet we now present microscopic and enzymatic evidence for a sulfide-based chemoautotrophic symbiosis in the class Gastropoda.

Materials and Methods

Specimens for this study were collected at a depth of approximately 3650 m at the "Snail Pit" site by the sub-

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Figure 1. Mariana gastropods encrusting an active hydrothermal chimney at 3632 m depth in the Mariana Back-Arc Basin. Large snails are approximately 5 cm in diameter. The temperature probe at top right measured temperature anomalies of 2–20°C where the snails live. Limpets, caridean shrimp, and brachyuran crabs are common associates of the snail.

mersible *Alvin* and transported in an insulated container to the surface where individual snails were either dissected and fixed for transmission electron microscopy (TEM) or frozen immediately in liquid nitrogen for enzyme assays. A morphological and taxonomic description of the snail is being prepared by S. Ohta and T. Okutani, Tokyo University of Fisheries, Tokyo 108, Japan.

Microscopy

Gill squash preparations for light microscopy were prepared by thawing approximately 50 mg of frozen tissue at room temperature in a sterile seawater solution containing 0.1 μg/ml of the specific DNA stain 4,6-diamidino-2-phenylindole (DAPI). The tissue was pressed under a glass cover slip and viewed under epifluorescence microscopy according to the methods of Porter and Feig (1980).

Tissues for transmission electron microscopy (TEM) examination were fixed freshly with 2% glutaraldehyde/0.5% formaldehyde in 75 m*M* cacodylate buffer (pH 7.4, 7.5% w/v sucrose). After rinsing with buffer, tissues were post-fixed in 1% osmium tetroxide, dehydrated in a

graded ethanol series followed by propylene oxide, then embedded in Epon 812. Thin sections were cut with a diamond knife, stained with lead citrate and uranyl acetate, and examined and photographed with a JEOL-100CX electron microscope.

Enzyme and elemental sulfur measurements

For enzyme assays and measurements of percent elemental sulfur, tissues frozen in liquid nitrogen for 2 to 5 weeks were transferred to a −80°C freezer where they were stored for approximately 3 weeks prior to analyses. We determined the metabolic potential of the bacterial endosymbionts by assaying for enzymes responsible for sulfur oxidation and subsequent ATP generation (ATP-sulfurylase [E.C. 2.7.7.4], APS-reductase [E.C. 1.8.99.2], rhodanese [E.C. 2.8.1.1], and for the ATP dependent fixation of carbon dioxide via the Calvin-Benson cycle (ribulose bisphosphate carboxylase (RuBPCase) [E.C. 4.1.1.39]). ATP-sulfurylase was measured with the method of Felbeck (1981), APS-reductase according to Peck *et al.* (1965), rhodanese according to Smith and Lascelles (1966), and RuBPCase according to Wishnick and Lane (1971). Activities of this suite of enzymes have been used in the past as a diagnostic tool to assess the chemoautotrophic potential of invertebrates containing putative sulfur-oxidizing symbionts. The ability of the snails to oxidize and thereby detoxify sulfide was determined by assaying for activity of "sulfide oxidase" with the method of Powell and Somero (1985). As a final determinate of sulfur metabolism, we measured an intermediate product of sulfur oxidation—elemental sulfur—in the gill and foot tissues of the snail using two methods—a spectrophotometric method of Schedel and Trüper (1980) as modified by Vetter (1985) and a gas chromatographic method of Richard *et al.* (1977) as modified by Fisher *et al.* (1987). We tested for methylotrophic symbionts by assaying for methanol dehydrogenase [E.C. 1.1.99.8] using the spectrophotometric method of Anthony and Zatman (1965).

Gill and foot tissues from five snails were each analyzed separately for RuBPCase, sulfide oxidase, ATP-sulfurylase and the spectrophotometric determination of percent elemental sulfur. Assays for rhodanese, APS-reductase, methanol dehydrogenase, and the gas chromatographic measurement of percent elemental sulfur were performed on tissues from different individuals. For all enzymes, except for methanol dehydrogenase, frozen trophosome tissue from the vestimentiferan tubeworm *Riftia pachyptila* was used as the positive control. Gill tissues of an unidentified mytilid, demonstrated to harbor methanotrophic symbionts (Childress *et al.*, 1986), served as the positive control for methanol dehydrogenase.

Results

Examination of dissected snails revealed that the gills were extremely hypertrophied, lobular in shape, light in color, and comprised approximately 40% of body volume. Viewed under epifluorescence microscopy, DAPI-stained gill-squash preparations contained abundant rod-shaped, membrane-bound bodies approximately 5 μm in length which fluoresced blue. When examined under polarizing light, these putative symbionts contained spherical, refractile inclusions reminiscent of the liquid crystalline sulfur inclusions described in the symbiotic sulfur bacteria of certain bivalves inhabiting sulfide-rich environments (Vetter, 1985). Indeed, the elemental sulfur content in the gills was comparable to that of the vent bivalve *Calyptogena magnifica* and in the trophosome tissue of the vestimentiferan tubeworm *Riftia pachyptila* (Table I).

TEM examination of thin transverse sections taken across gill filaments revealed that the putative symbionts contained no membrane-bound organelles and possessed a non-membrane-bound nuclear region thus supporting the view that they are prokaryotic. The bacteria were packed densely within microvilli-fringed cells (Fig. 2), resembling closely the arrangement of bacteria within the bacteriocyte cells of symbiont-containing bivalve mollusc gills (Reid and Brand, 1986).

We found activities of ATP-sulfurylase, rhodanese, RuBPCase, and sulfide oxidase in gill homogenates of the Mariana vent snail comparable to levels reported for *C. magnifica* and for *R. pachyptila* although no significant activity of APS-reductase was detected with the methods used (Table I). With the exception of the low activity of sulfide oxidase, snail foot tissue and control assays without respective substrate showed no activity for any of these enzymes. Methanol dehydrogenase activity was not detected.

Discussion

The presence of bacteria within specialized gill cells in the Mariana gastropod coupled with the activities of enzymes responsible for fixing carbon dioxide and extracting energy from sulfide represent the first documentation of chemoautotrophy in the class Gastropoda. Comparable morphology and enzyme levels have been described previously only in symbiont-bearing bivalves and tubeworms which have been demonstrated to fix carbon dioxide into metabolites of intermediary metabolism (Felbeck, 1983, 1985). Another vent gastropod, an unde-

Table I

Percent elemental sulfur (dry weight) and enzyme activities in tissues of the Mariana gastropod *and other hydrothermal vent taxa*

	Mariana gastropod (n = 5)		*Calyptogena magnifica* (Bivalvia)	Riftia pachyptila (Vestimentifera)
	Gill	Foot	Gill	Trophosome
Elemental sulfur				
a) spectrophot.	4.4 ± 1.4	N.D.	NA	NA
b) gas chrom.	2.8 ± 1.0§	N.D.	4.4*	13.5*
ATP-sulfurylase	42.6 ± 8.4	N.D.	2.5#	74**
APS-reductase	N.D. (n = 2)	N.D.	NA	23.3**
Rhodanese	25.9 (n = 2)	N.D.	NA	7.6**
RuBPCase	0.2 ± .02	N.D.	0.4**	0.22**
Sulfide oxidase	5.3 ± 1.4	0.4 ± 0.3	6.1 ± 2.1 (n = 22)##	31.7 ± 8.8 (n = 8)##
MeOH dehydrog.	N.D. (n = 2)	NA	N.D.	N.D.

Elemental sulfur measurements were determined on freeze-dried tissues. Enzyme activities are given in international units (μmoles of substrate converted to product per min) per g wet weight ± standard deviation (where applicable). N.D.: not detectable; NA: not available. Detection levels (in units per liter of extract) for "N.D." enzymes are: ATP-sulfurylase (10), APS-reductase (0.01), Rhodanese (5), RuBPCase (50), and MeOH dehydrog. (25).

§ n = 5.

* J.J.C., pers. obs.

** Felbeck *et al.*, 1981.

H.F., pers. obs.

Fisher and Childress, 1984.

scribed limpet from the Juan de Fuca Ridge, has been shown to endocytose bacteria that colonize the gill epithelium (deBurgh and Singla, 1984). However, in this case the bacteria are not contained within bacteriocyte cells but appear to be immediately degraded by lysosome-like cells and, therefore, are probably not involved in a symbiotic association with the limpet.

The proportion of the Mariana gastropod's metabolism that is fueled by symbiont-derived carbon is not yet known, for they also possess an apparently functional radula and gut although no individuals examined thus far contained a significant amount of material within their gut. Sacoglossan gastropods of the family Elysiidae also maintain a functional radula and gut yet derive most of their energetic requirements from photosynthetically fixed carbon of functional chloroplasts acquired from ingested algae (Trench, 1975). Further, the tight clustering of the snails around the vents, the greatly enlarged and modified gill, and the high enzyme activities relative to those expected in a strictly heterotrophic organism, in combination suggest that the snails derive nutritional or energetic benefit from the symbiosis. The snails may alternatively rely on their symbionts principally for detoxification of sulfide thus allowing them to graze upon epilithic bacteria closer to the vents. However, gastropods that graze on sulfur bacteria at shallow-water vents (Stein, 1984) do so without benefit of sulfide detoxification by bacterial symbionts.

Although the oxidation of sulfide results directly in ATP synthesis by the bacteria of *Riftia pachyptila* (Powell and Somero, 1986a), and in the mitochondria of the gutless protobranch clam, *Solemya reidi* (Powell and Somero, 1986b), sulfide is also a potent respiratory inhibitor whose entrance, transport, and metabolism—even in vent organisms—must be tightly controlled (Powell and Somero, 1986b; Vetter *et al.*, 1987). The low level of sulfide oxidizing activity in the snail's foot tissue relative to the gill suggests that "sulfide oxidase" activity may be restricted to the superficial layers of the foot as is found in *S. reidi* (Powell and Somero, 1986a) or that the animal tissue may be protected by another defense mechanism, perhaps a circulating sulfide binding protein as in the blood of *R. pachyptila* (Arp and Childress, 1983; Fisher and Childress, 1984). However, equilibrium dialysis of the gastropod blood revealed no such binding activity.

Also indicative of the prokaryotic metabolism of sulfide is the high level of elemental sulfur in the snail's gill. It has been suggested that elemental sulfur stored by bacteria within the gills of symbiont-containing bivalves represents an intermediate product of sulfide oxidation that may serve as an energy storage reserve which can be oxidized to produce energy in the absence of ambient sulfide (Vetter, 1985). Since deep-sea hydrothermal vents can be locally ephemeral features (Grassle, 1986), such an energy reserve might benefit a motile gastropod which

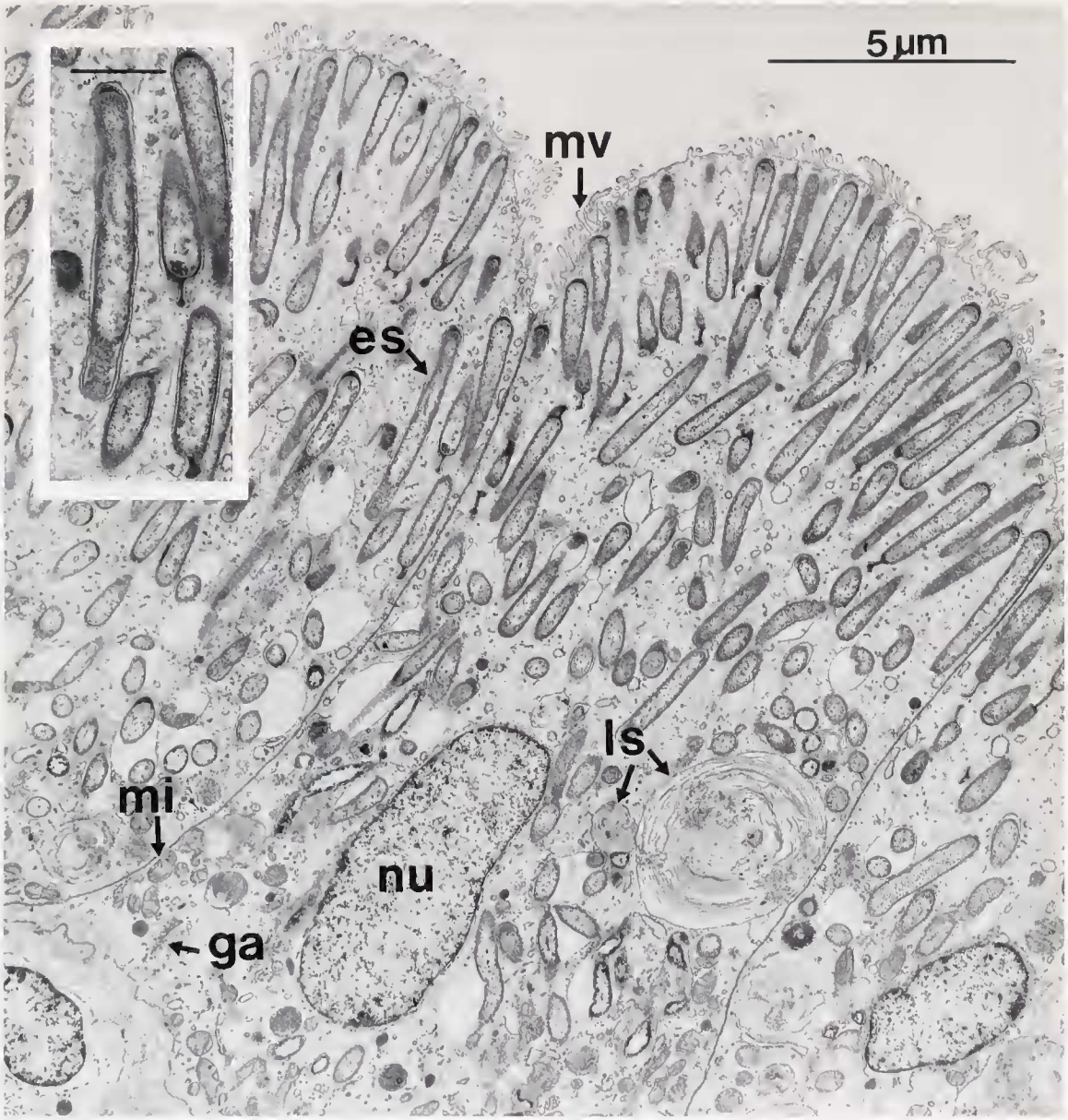

Figure 2. Transmission electron micrograph showing rod-shaped endosymbiotic bacteria in the bacteriocytes of the gill of the Mariana gastropod. Nuclei of the bacteriocytes (nu), lysosomes (ly), mitochondria (mi), and Golgi apparatus (ga) are found in the basal regions of the cells while the slender endosymbionts (es) are typically observed in the outer region of the cells arranged in a radial fashion. The outer surface of the bacteriocyte is covered with microvilli (mv). *Inset.* Enlargement of endosymbionts showing cell membranes (scale bar = 1 µm.).

could find more suitable habitat in the face of diminishing vent flow.

Endosymbioses between sulfur bacteria and bivalve molluscs have been described worldwide from deep-sea hydrothermal vents, sewage outfalls, mangrove swamps, and other environments where there is simultaneous access to reduced sulfur compounds and molecular oxygen (Cavanaugh, 1983; Felbeck, 1983; Schweimanns and Felbeck, 1985). Although gastropods occur in many of these habitats, and large gastropod shells have been described at hydrothermal vents in the Manus Back-Arc basin near New Guinea (Both *et al.*, 1986), none until now have been reported to contain bacterial endosymbionts. From evolutionary and biogeographical stand-

points, it is of interest that gastropod endosymbiosis has apparently not attained the wide distribution reported in bivalves and has not been reported outside the western Pacific. Nonetheless, the chemoautotrophic potential of the Mariana gastropod suggests that additional examples of similar symbioses within this and other taxa may yet be found.

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